REMARKS

Claims 21, 22, and 25-44 are pending in the present application.

The rejection of Claims 21, 22 and 25 under 35 U.S.C. §102(b) over Dahl et al is respectfully traversed.

Dahl et al disclose a method for culturing and manipulating cells derived from synovial specimens (synovial cavity) which included the synovium (e.g., the synovial lining; see Patients and Methods on page 647-650). At no point do Dahl et al disclose or suggest isolating, culturing, or manipulating cells derived from synovial (joint) *fluid* as the Examiner alleges.

It should be further noted that Dahl et al disclose the characterization of the cells isolated from rheumatoid synovial *tissues* in the section entitled "Cell Characterisation" in the Results heading on page 650. In this section Dahl et al disclose that "the preparations showed a heterogeneous cell population with small rounded cells, non-dendritic macrophage-like cells and dendritic cells", "a very few fibroblast-like, elongated cells were seen" and "they (the cells) were appeared to be highly esterase positive". Furthermore, they obtained the cells, which had the general appearance of fibroblasts. At no point do Dahl et al disclose that they obtained osteoclast precursor cells.

In contrast, in the presently claimed invention the method for culturing cells derived from joint fluid, not tissue or lining, to produce osteoclast precursor cells. Accordingly, the product obtained by the claimed method are osteoclast precursor cells and not "small rounded cells, non-dendritic macrophage -like cells and dendritic cells" or "a very few fibroblast-like, elongated cells." Additionally, it is well known in the art that osteoclast precursor cells appear to be esterase negative.

In view of the foregoing, Applicants submit that Dahl et al fails to disclose the invention as claimed and, therefore, the present invention is not anticipated by this disclosure. As such, withdrawal of this ground of rejection is requested.

The rejection of Claims 42-44 under 35USC §103(a) over Purton et al taken with Pollice et al is obviated in part by amendment and traversed in part.

In the outstanding Office Action the Examiner confirms that the culturing protocol for producing osteoclast precursor cells as described by Purton et al does not contain a step of eliminating non-adherent cells after 1-2 hours of culturing in the medium (Office Action, page 5, line 11-13). Apparently in an attempt to compensate for this deficiency the Examiner cites Pollice et al noting that this reference teaches that 2-hour cell preplating or removal of non-adherent cells after incubation for 2 hours decreases the number of TRAP negative cells in population of mononuclear cells derived *from bone marrow* as intended isolation and for producing osteoclast precursor cells (Office Action, page 5, line 14-17).

However, Applicants submit that the method of Pollice et al is fundamentally different from the presently claimed invention or that disclosed by Purton et al. Specifically, Pollice et al use cells derived from the femoral shafts (bone marrow). In the bone marrow, there are many kinds of cells containing osteoclast precursor cells. In the method of Pollice et al, they had osteoclast precursor cells from scratch, decreased the number of acid phosphatase-negative cells in culture by preplating for 20 minutes and distinguished between the adherent cells (osteoclast precursor cells) and non-adherent cells by incubation for 2 hours to isolate the osteoclast precursor cells. Therefore, their preplating and 2-hour culturing is not for differentiation to osteoclast precursor cells *but for decreasing the number of acid phosphatase-negative cells and isolating osteoclast precursor cells since bone*

marrow as a starting material contains osteoclast precursor cells. For the Examiner's convenience the method of Pollice et al is summarized in the right column of the flow chart **submitted herewith**.

In contrast, in the present invention Applicants use cells derived from peripheral blood. It is well known that the peripheral blood *does not contain osteoclast precursor cells* although it contains monocytes, which differentiate to osteoclast precursor cells. In the method of the present invention, Applicants cultured the cells (the PBMCs) for 1-2 hours before removing the non-adherent cells in a medium. In other words, culturing at this stage is just for distinguishing between adherent cells (monocytes) and non-adherent cells.

After rinsing out non-adherent cells, the obtained monocytes were cultured in a medium for several weeks. During the culturing, the monocytes were differentiated to osteoclast precursor cells or the other cells (including the acid phosphatase-negative cells) and then, the osteoclast precursor cells were isolated because all cells except preosteoclasts died out and only preosteoclasts survived (Page 18, line 14 of the present specification). *Therefore, the culturing after rinsing out non-adherent cells is necessary to differentiate from monocytes to osteoclast precursor cells and isolate osteoclast precursor cells from said obtained cells.* For the Examiner's convenience the method of the present invention is summarized in the left column of the flow chart **submitted herewith**.

As will be appreciated from the foregoing, the preplating and 2-hour incubation in the method of Pollice et al is ***not*** equivalent to any step in the claimed method because Pollice et al fail to disclose a step to obtain monocytes and to differentiate from monocytes to osteoclast precursor cells. They just disclosed how to isolate osteoclast precursor cells from miscellaneous cells including osteoclast precursor cells.

Similarly, Purton et al disclose a method in which non-mobilized peripheral blood is subjected to hemolysis followed by culturing for 3 days. Subsequently, the non-adherent cells are removed to produces a sample containing PBMC with cytokine(s). After rinsing out non-adherent cells, the obtained PBMCs were cultured in a medium for several weeks. During the culturing, the PBMCs were differentiated to single large, multinucleated, TRAP-positive cells (i.e., osteoclasts). *Therefore, the culturing after rinsing out non-adherent cells is necessary to differentiate from PBMCs to osteoclasts.* For the Examiner's convenience the method of Purton et al is summarized in the center column of the flow chart **submitted herewith.**

In view of the foregoing differences in the disclosures of Pollice et al and Purton et al, Applicants submit that one having ordinary skill in the art would have no motivation to modify Purton et al's protocol by adding step from Pollice et al's protocol. Applicants remind the Examiner that the mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art *also suggests the desirability of the combination* (MPEP §2143.01). In this case, no such motivation can be found in Pollice et al and Purton et al.

Furthermore, in the Examiner's "Response to Arguments" the Examiner appears to misunderstand the arguments presented in the response filed on September 8, 2005. In the Office Action, the Examiner states "Applicants appear to argue that the method of Purton et al results in production of osteoclasts but not in production of preosteoclasts because cells are TRAP-positive" (Office Action, Page 6, line 19-20). However, Applicants note that on page 7, lines 11-12 of the response filed on September 8, 2005, it is stated in relation to Purton et al that "their TRAP-positive cells were osteoclasts *because those cells are multinucleated.*"

Osteoclast precursor cells are single nucleated and osteoclasts are multinucleated. Therefore, Purton et al did not disclose they obtained osteoclast precursor cells.

To emphasize this distinction and to further differentiate the present invention from the disclosure of Purton et al, Applicants have amending Claim 42 to specify that the claimed method is a "method for producing an osteoclast precursor cell *without producing osteoclasts*." With respect to the term "without producing osteoclasts," Applicants submit that page 12, lines 16-21 and page 18, lines 10-18 clearly state that "During the culture period, all cells except preosteoclasts died out, and only preostetoclasts survived." Thus, the claimed method produces osteoclast precursor cells (i.e., preostetoclasts) without producing osteoclasts and this amendment finds support therein.

Purton et al did not disclose how to get the osteoclast precursor cells without producing osteoclasts, although Purton et al disclose the method for obtaining both the osteoclasts precursor cells and osteoclasts when they used G-PBMC (Fig. 3, page 1804). In the method of the present invention, Applicants cultured the cells for 1-2 hours before rinsing out the non-adherent cells in a medium. The step is necessary to obtain monocytes from peripheral blood mononuclear cells. However, if the time for culturing is too long, some cytokines, which stimulate osteoclast precursor cells, are produced.

Applicants submit that in 1-2 hours of culturing non-adherent cells did not produce any cytokines, which stimulate osteoclast precursor cells, therefore, osteoclast precursor cells differenciated from monocytes in culturing after rinsing were not stimulated by any cytokines and osteoclast precursor cells may be obtained *without osteoclasts* (Testing example (1) on page 19, and Figure. 1).

However, in the method of Purton et al, the cells were cultured with non-adherent cells for 3 days. 3 days are long enough for the non-adherent cells to produce various

cytokines, which can stimulate osteoclast differentiation. Purton et al failed to disclose the culture time needed to obtain monocytes without producing cytokines which can stimulate osteoclast precursor cells and keep them before differentiating to osteoclasts. Even if Purton et al shows that osteoclasts can be differentiated from peripheral blood, they fail to disclose when the culturing of peripheral blood mononuclear cells should be stopped and how osteoclast precursor cells can be obtained without producing osteoclasts.

Additionally, in the method of Pollice et al, they had osteoclast precursor cells from the onset and isolated the osteoclast precursor cells. Therefore, they also fail to teach when the culturing of peripheral blood mononuclear cells should be stopped to obtain monocytes without producing various cytokines and how osteoclast precursor cells can be obtained from the monocytes without producing osteoclasts.

In view of the foregoing, Applicants submit that the method of Claims 42-44 would not be obvious in view of the combined disclosures of Purton et al and Pollice et al. Withdrawal of this ground of rejection is requested.

The rejection of Claim 44 under 35USC §112, second paragraph, is obviated by the present amendment.

Applicants note that Claim 44 has been amended to clearly indicate "what cells are cultured."

In view of the present amendments, Applicant request withdrawal of this ground of rejection.

Applicants submit that the present application is in condition for allowance. Early notification to this effect is respectfully requested.

Respectfully submitted,

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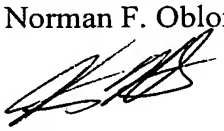

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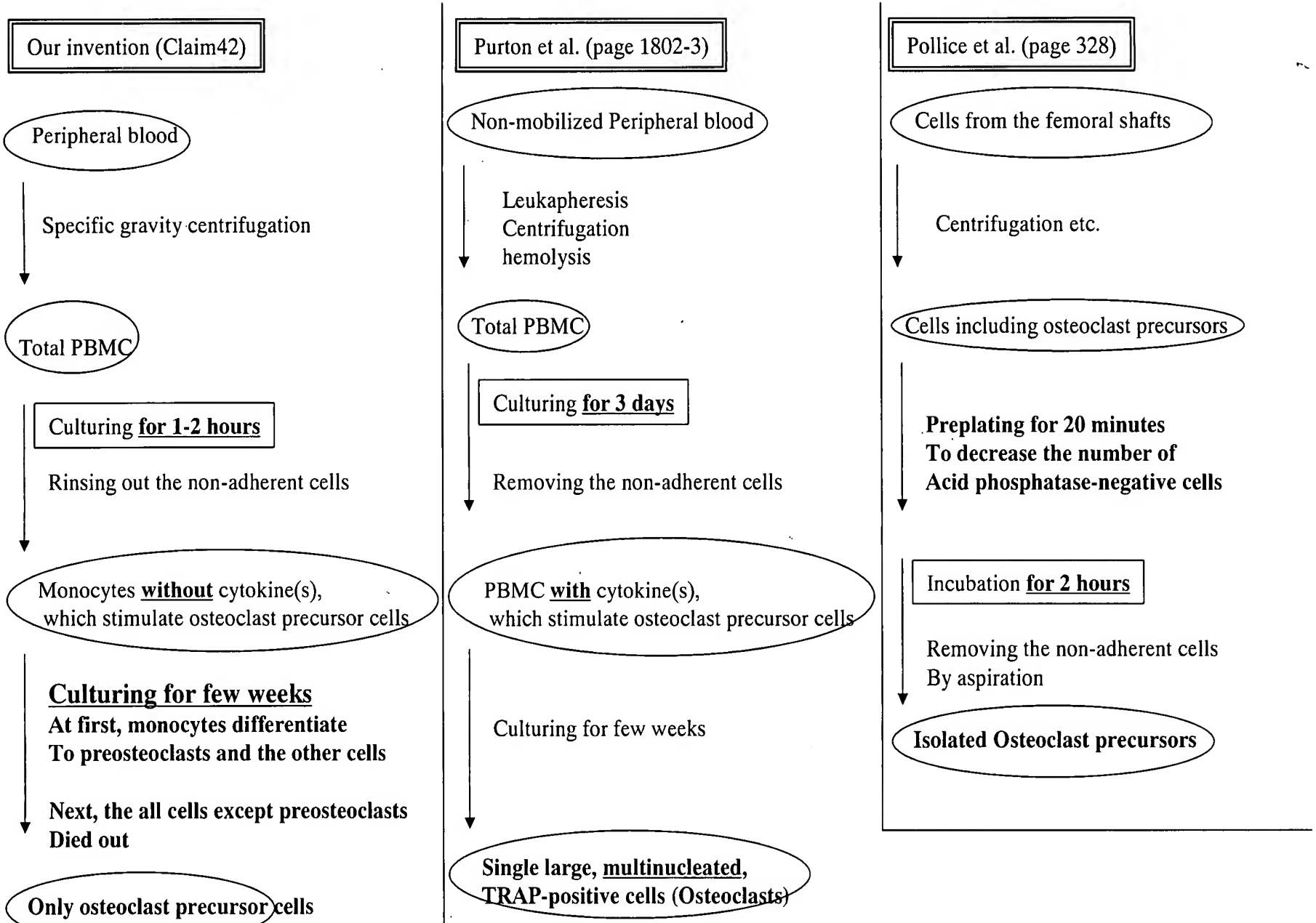

** When they used G-PBMC, they obtained osteoclasts, preosteoclasts and the other cells. (page 1806, RESULTS)